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THE EFFECT OF COPPER, ZINC, IRON AND LEAD SALTS ON AMMONIFICATION AND NITRIFICATION IN SOILS

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In the course of investigations on the effect of smelter wastes on crop growth one of the writers decided to test also the effects of the salts of the heavy metals on the transformation of organic nitrogen in the soil into ammonia and nitrates which serve as the source of nitrogen for plants. This correlation between the effects of external factors upon both the soil flora and the physiological condition of plants was deemed eminently worth while, for reasons which are at once obvious to the careful student of soil fertility problems. Some of the results obtained with plants have been published,[1] and still fuller data dealing with the same problem will soon find publication elsewhere. In view of the foregoing the writers have carried out experiments dealing with the toxic and stimulating effects of copper, zinc, iron and lead on the ammonifying and nitrifying flora of a sandy soil. The results, owing to their interesting nature and their cogency at this time are here given apart from the results obtained with plants.

METHODS OF THE EXPERIMENTS

The work here described was carried out by the direct soil-culture method in a manner fully explained by one of us elsewhere.[2] No attempt was made to modify the soil flora, but the

[1] Bot. Gaz., vol. 55, p. 409.
[2] Cent. für Bakt., 2te Abt., vol. 32, p. 58, vol. 33, p. 305.

soil with its natural flora was employed. The ammonia determinations were made in accordance with methods described in the papers above cited and the nitrate determinations in accordance with the method described by Burgess.[3]

The work carried out by other investigators dealing with the subject in hand has thus far been rather meager and but few of the results possess pertinence with respect to our findings. In studying the amounts of copper in vineyard soils due to accumulation from copper-containing sprays, Prandi[4] was unable to note any damage to vines after the copper sprays had been used for a number of years. Moreover, he did not adjudge dangerous the amounts of copper which had accumulated in the soils studied in the quantities in which he found it there. He does, however, make the further interesting speculative statement that copper may have an important influence on the soil organisms. The original work of Eickmeyer[5] is unfortunately not accessible to us and, while it may contain some of the most cogent information on our subject which is in print, we can ascertain only that the investigator named studied the effects of copper and iron sulfates, among other poisons, on soil bacteria. In studying the bacteriology of ammonium compounds Ehrenberg[6] observes that the cause of the difference in the effects of ammonium compounds used for fertilizers in zinc pots and in the field must be that the zinc exercises a deleterious effect on "ammonia-fixing" bacteria which compete with the plants for soluble nitrogen in the soil. Owing to that, he believes ammonium fertilizers stimulate plants in zinc pots but have no effect in the field. Among studies on the effects of the poisonous metals here considered, on some of the lower organisms, may be mentioned those of Latham,[7] who obtained stimulation with low concentrations of $ZnSO_4$ for *Aspergillus niger*, and those of Remy and Rosing,[8] which indicate a marked stimulating effect of iron on Azotobacter organisms.

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3 Univ. Calif. Publ. Agr. Sci., vol. 1, no. 4, p. 51.

4 Staz. Sper. Gr. Ital., vol. 40, p. 531, cited from E. S. R., vol. 19, p. 755.

5 Winner Landw. Ztg., vol. 57, p. 600, cited from E. S. R., vol. 19, p. 625.

6 Fuhling's Landw. Ztg., vol. 27, p. 449.

7 Cited from E. S. R., vol. 21, p. 421.

8 Cent. für. Bakt., 2te Abt., vol. 30, p. 349.

The only work with which the writers are acquainted which allows of any direct comparison with our results is included in the splendid series of investigations of Fred[9] on the effects of small or limited quantities of various poisons on the higher as well as the lower plant organisms. In addition to showing the stimulating powers of certain poisons like CS_2 and $\left.\begin{matrix}C_2H_5\\C_2H_5\end{matrix}\right\rangle O$ on the bacterial count, nitrogen-fixing power, etc., of soils in direct soil cultures, that investigator gives evidence also of work done with the higher plants yielding similar results. Of a series of metallic salts, further, with which Fred worked he was unable to demonstrate stimulation to nitrification except perhaps one case of slight stimulation through the use of $FeSO_4$ (100 mgs. per 100 grams of soil). The salts tested were $MnSO_4$, $FeSO_4$, $CuSO_4$, and NaCl. The nitrifiable material employed was $(NH_4)_2SO_4$ and not the organic nitrogen of blood which we used.

Aside from these investigations, only one or two of which have any direct bearing on our subject, but little has been accomplished of either direct or indirect applicability to our results. Where any studies were carried out dealing with the effects of the heavy metals on bacteria, such as those of Kellerman and Beckwith[10] or Jackson,[11] they were prosecuted in solutions and therefore, as one of us has emphatically shown in other publications,[12] are scarcely comparable with results from soil cultures such as are below discussed, or with results obtained with plants in soil cultures.

Since the experiments below described were completed there has appeared from the pen of Greaves[13] a paper bearing on the effects of arsenic on ammonification and nitrification in soils. This work, because of the methods employed, is of pertinence here, and interesting, because of the stimulating powers of considerable quantities of arsenic in soils to ammonia and nitrate production by the natural flora. While arsenic, chemically considered, is a

[9] Cent. für Bakt., 2te Abt., vol. 31, p. 185.
[10] Bull. 100, Bur. Pl. Ind., U. S. D. A.
[11] Jour. Am. Chem. Soc., vol. 27, II, p. 675.
[12] Bot. Gaz., vol. 55, p. 409 and literature there cited.
[13] Cent. für Bakt., 2te Abt., vol. 39, p. 542.

totally different substance from the metals we have studied, it is interesting to compare its effects on the soil flora with those exercised by copper, zinc, lead and iron. This is especially so since arsenic so frequently occurs with the other elements in soils and in smelter wastes from which it may be transported to agricultural soils.

In closing this brief review of investigations dealing with the subject of this paper or one allied thereto, it should also be added that some fragmentary information was obtained in the course of other investigations by J. G. Lipman and his associates[14] which refers to the effects of $CuSO_4$, and $FeSO_4$ among other compounds on the ammonifying flora of the soil. These results indicate that copper stimulates ammonification very slightly at a concentration of 0.1 per cent, that $ZnSO_4$ gives no stimulation and is toxic at all concentrations, and that $FeSO_4$ at the concentration of 1 mg. per 100 grams of soil gives marked stimulation.

The Ammonification Experiments

To a fifty-gram portion of dry soil, one gram of tankage (9.62% N) was added and thoroughly mixed with it. Sterile distilled water was then added, to make optimum moisture conditions, and also varying quantities of the salts to be tested. The soil, water and salt mixture was thoroughly stirred in the tumbler, the latter covered with a Petri dish cover and incubated for one week at 27° C to 30° C. The results of the ammonia determinations at the end of the incubation period, together with other necessary explanatory data, will be found in Table 1. The sulfates of the metals tested were employed throughout. It will be noted that the results of duplicate determinations are given throughout, averages being omitted since the reader can so readily ascertain them from the data submitted.

It is at once obvious, from even a casual examination of the data given in Table 1, that none of the metals employed exercises a stimulating effect on the ammonifying flora of the soil employed even at the smallest concentrations (50 p.p.m.). Despite that

[14] N. J. Exp. Sta. Bull. no. 246, p. 32.

TABLE 1

THE EFFECT OF CU, ZN, FE, AND PB ON AMMONIFICATION IN SOILS

No.	$CuSO_4$ % of dry soil	N as NH_3 produced mgs.	$ZnSO_4$ % of dry soil	N as NH_3 produced mgs.	$FeSO_4$ % of dry soil	N as NH_3 produced mgs.	$PbSO_4$ % of dry soil	N as NH_3 produced mgs.
1	0	40.60	0	40.60	0	40.60	0	40.60
2	0	39.29	0	39.20	0	39.20	0	39.20
3	.005	40.32	.005	36.82	.005	39.34	.005	38.50
4	.005	38.50	.005	36.68	.005	37.94	.005	37.94
5	.010	36.40	.010	39.20	.010	39.48	.010	37.52
6	.010	lost	.010	37.38	.010	37.38	.010	38.78
7	.025	35.00	.025	36.82	.025	37.24	.025	41.58
8	.025	35.70	.025	35.98	.025	lost	.025	39.90
9	050	35.98	.050	33.74	.050	37.10	.050	40.32
10	.050	36.26	.050	32.20	.050	39.20	.050	38.78
11	.075	32.20	.075	31.50	.075	36.68	.075	39.76
12	.075	30.80	.075	31.92	.076	37.94	.075	38.92
13	.100	29.96	.100	31.08	.100	38.22	.100	35.70
14	.100	28.00	.100	29.12	.100	39.34	.100	35.84
15	.125	27.58	.125	31.50	.125	35.98	.125	35.00
16	.125	28.84	.125	31.50	.125	34.58	.125	35.42
17	.150	28.70	.150	34.30	.150	35.56	.150	33.04
18	.150	29.96	.150	33.74	.150	37.52	.150	34.72
19	.200	26.66	.200	34.58	.200	36.40	.200	35.00
20	.200	27.44	.200	33.60	.200	37.10	.200	32.90
21	.250	28.70	.250	32.90	.250	36.68	.250	35.00
22	.250	27.58	.250	32.06	.250	37.52	.250	36.26

fact, however, the toxicity of no one of them is very marked. While this latter observation is in accord with the findings of J. G. Lipman above referred to, the former result is entirely at variance with them. It is significant to remark in this connection, however, that the agreement between duplicate determinations was on the whole much better in our experiments than in those carried out at the New Jersey Experiment Station and should therefore render our results more trustworthy. Indeed, as the authors of the New Jersey bulletin state, for their work "there is not sufficient evidence upon which to base conclusions."

Comparing the relative toxicities of the salts tested by us it would appear that copper is the most toxic, zinc the next, lead the next, and iron the least toxic of the four sulfates. This assertion is based on the fact that at the highest concentrations at which it was used, copper depresses ammonification by about

30 per cent, zinc by 20 per cent, lead by about 15 per cent, and iron by only about 12 per cent of that of the normal yield. Despite this fact, however, copper begins to manifest its toxicity most slowly of the four metals, since it shows little if any toxicity at a concentration of .005 per cent, whereas all the other metals show clearly a slight toxic effect even at that concentration. Just why this peculiar relationship between the metals tested and the ammonifying flora should obtain is by no means easy of explanation.

Speaking of the data in Table 1, by and large, it seems justifiable to venture the assertion that all the metals become markedly more toxic at concentrations of 0.1 per cent of the dry weight of the soil or in concentrations in excess of that amount. Other details worthy of mention here are the fact that at times the depressing effect of a certain concentration is followed by a stimulating effect at an increased concentration of the same metallic salt. Also, the very striking fact is noted of the relatively slight increases of toxicity which accompany relatively large increases in concentration of the salt. The latter is particularly marked in the case of $FeSO_4$. Whether or not this latter result is to be explained by the differences in solubility which obtain between the different metallic sulfates employed, as well as by the different adsorptive power exercised by the soil towards every one of them, still remains to be shown.

In general, it is worthy of special remark here that though these so-called very toxic metals manifest undoubted toxicity towards the ammonifying flora, and no stimulation in any concentration of them, the toxic effect is relatively small and in some cases even absolutely slight.

The Nitrification Experiments

To 100-gram portions of sandy soil in tumblers were added two grams of dried blood (13.29% N), enough water to make optimum moisture conditions, and the necessary amounts of the salts to be tested. The mixture was prepared for incubation as above described for the ammonification cultures and incubated

for four weeks at 27° C to 30° C. Other explanatory data are given in Table 2, which shows the results obtained from the nitrate determinations made as above explained.

TABLE 2

THE EFFECT OF CU, ZN, FE, AND PB ON NITRIFICATION IN SOILS

No.	$CuSO_4$ % of dry soil	Nitrates produced mgs.	$ZnSO_4$ % of dry soil	Nitrates produced mgs.	$FeSO_4$ % of dry soil	Nitrates produced mgs.	$PbSO_4$ % of dry soil	Nitrates produced mgs.
1	0	10.64	0	11.20	0	11.20	0	11.20
2	0	9.80	0	10.64	0	10.64	0	10.64
3	.0125	7.84	.0125	10.36	.0125	14.56	.0125	5.60
4	.0125	10.64	.0125	11.48	.0125	16.24	.0125	7.00
5	.0250	11.20	.0250	11.20	.0250	12.60	.0250	9.52
6	.0250	10.08	.0250	12.60	.0250	12.88	.0250	8.68
7	.0500	17.64	.0500	16.24	.0500	13.72	.0500	10.08
8	.0500	18.76	.0500	17.36	.0500	13.44	.0500	11.20
9	.0750	24.36	.0750	21.00	.0750	14.00	.0750	11.48
10	.0750	24.92	.0750	23.80	.0750	11.76	.0750	14.00
11	.1000	23.80	.1000	23.52	.1000	8.40	.1000	7.56
12	.1000	24.64	.1000	22.68	.1000	7.84	.1000	7.56
13	.1250	21.00	.1250	17.08	.1250	18.20	.1250	11.20
14	.1250	19.32	.1250	17.22	.1250	21.00	.1250	12.88
15	.1500	20.44	.1500	17.08	.1500	20.72	.1500	6.44
16	.1500	19.88	.1500	17.36	.1500	19.60	.1500	6.72

It is quite clear from the foregoing table that all of the salts used exercise marked effects on the nitrifying flora of the soil employed. For the reason that some pronounced differences obtain between the action of the different salts we shall discuss each of them separately at first.

$CuSO_4$

It can be seen at a glance that the data in Table 2 show copper to be the most stimulating of the salts employed so far as a soil-nitrifying flora is concerned. While, unfortunately, the duplicate determinations for the lowest concentration do not agree well, they indicate clearly that in those quantities (0.0125 per cent, or 125 parts per million) copper has either no effect on the nitrifying flora or a slightly depressing one. By doubling the concentration of the copper salt we obtain a slightly stimulating effect, but by doubling the latter concentration we obtain

a most striking stimulating effect on the nitrifying flora, which results in the production of nearly twice as much nitrate nitrogen as is yielded in the normal soil receiving no copper at all. But the stimulation of the nitrifying flora does not cease at a concentration of $CuSO_4$ equivalent to 0.05 per cent of the dry weight of the soil. Even at a concentration of 0.075 per cent of $CuSO_4$ we obtain stimulation and one-third again as much nitrate as is produced when copper is present at the preceding concentration (0.05 per cent $CuSO_4$). At 0.1 per cent $CuSO_4$ about the same amount of nitrate is produced as at a concentration of 0.075 per cent, or perhaps slightly less, and at the highest concentration of $CuSO_4$ employed, namely 0.15 per cent, we obtain an increase of about 100 per cent of nitrate over the normal soil to which no copper is added. That such remarkable stimulation should be exercised by $CuSO_4$ for the nitrifying flora and at such high concentrations of that supposedly toxic salt is a new and interesting fact of great significance.

While our results agree with those of Fred above cited as regards the lower concentration of $CuSO_4$ which he employed, namely 0.01 per cent, they are wholly at variance with his results as regards his only other concentration, 0.1 per cent $CuSO_4$. The latter gave Fred no stimulation for the nitrifying flora whatever, but it yields us nearly the maximum point of stimulation of $CuSO_4$ for the flora in question, and in a series of results in which the existence of stimulation is throughout remarkable. To what circumstance these differences between our work and that of Fred are attributable is not readily determined. It can scarcely be due to the difference in the strain or vigor of the nitrifying flora, for we have obtained similar results to those above described with widely different soils which were employed in corresponding vegetation experiments. That the nitrifiable materials were wholly different may, however, be a factor of considerable significance. Not only because of its totally different physical nature, but because of possible antagonistic effects which it might induce, $(NH_4)_2SO_4$ might well be expected to yield different results in nitrification work from those obtained with dried blood or similar forms of nitrogen, especially when salt effects are studied.

A question more difficult of solution is that involving the cause or causes of the stimulating effects of $CuSO_4$ on the nitrifying flora or the production of nitrates in soils. The experiments of Fred have included such as aimed at the discovery of the reply to the question just raised. The results of such experiments, however, would seem to throw no positive light on its solution. Two factors do seem, nevertheless, to have been eliminated. But little support was found for the idea that poisons like the salts above employed destroy soil toxins which might interfere with bacterial efficiency, as well as for the further idea that the effect of such poisons in the destruction of amoebae might manifest itself by a stimulation for the soil organisms. It seems, to use Fred's expression, to be a "Reizwirkung" on the part of the salts and other poisons added to the nitrifying flora, but the question as to how this "Reizwirkung" is accomplished still remains unanswered. This question is further discussed below.

$ZnSO_4$

Zinc evidently exercises relatively the same influence on the nitrifying flora as copper. Like the latter, it stimulates but very slightly at a concentration of 0.0125 per cent $ZnSO_4$, does so very definitely at a concentration of 0.025 per cent, very markedly at 0.05 per cent, and still more markedly at concentrations of 0.075 per cent and 0.10 per cent, at which latter the highest point of stimulation is reached. Concentrations in excess of 0.1 per cent $ZnSO_4$ seems to be much less stimulating to nitrate production than similar amounts of $CuSO_4$. The absolute amounts of nitrate produced by the nitrifying flora under the stimulus of zinc salts seem to be throughout slightly smaller than those produced by similar flora in the presence of $CuSO_4$.

$FeSO_4$

With $FeSO_4$ we obtain effects on the nitrifying flora which possess many characteristics different from those obtained with $CuSO_4$ and $ZnSO_4$. In the first place, stimulation is marked with the smallest concentration, 0.0125 per cent $FeSO_4$, while it is either extremely slight or wanting with the other salts at

similar concentrations. While stimulation continues up to and including concentrations of 0.075 per cent, it is not so great as with the smallest concentration, and increases or decreases with the different amounts of $FeSO_4$ employed, with no regularity. Then, beyond the concentration of 0.075 per cent $FeSO_4$, a wholly inexplicable sudden toxicity manifests itself, and stranger still, beyond that point with two additional and larger concentrations employed we obtain the most marked stimulation in the whole series of cultures and about equivalent to the stimulation effected by $CuSO_4$ at similar concentrations.

The causes of this erratic behavior of $FeSO_4$ are a mystery as yet. We suggest that the rapidity of oxidation of the $FeSO_4$ may vary and so the ferrous and ferric salts may yield different effects, though this explanation does not appear wholly satisfactory. The distribution of the salt in the soil culture may also be a factor. What appears to us as another promising explanation for the behavior of the $FeSO_4$ under discussion is its effect on the physical condition of the soil and the modification of the latter's absorptive power for moisture and gases. Whether or not the effects noted may be related to transformations in the amounts of available bacterial foods present cannot be answered from the data in hand.

$PbSO_4$

In this series of cultures we are confronted with effects which are in certain definite ways different from either those of $CuSO_4$ and $ZnSO_4$, on the one hand, and $FeSO_4$ on the other hand. Lead is the only one of the metals used above which exercises an unquestionable and marked toxicity on the nitrifying flora at even the lowest concentration (0.0125 per cent $PbSO_4$). But while that is so, larger amounts of $PbSO_4$ make conditions in the soil more favorable for nitrification as the lead salt increases in concentration from 0.0125 per cent to 0.05 per cent, at which point nitrification is about as active as in the normal soil. When, however, the $PbSO_4$ is still further increased we obtain what appears to be a definite stimulation to nitrification. Oddly enough, however, at a concentration of 0.1 per cent, $PbSO_4$

behaves like a similar concentration of $FeSO_4$ to the nitrifying flora and becomes about as toxic as 0.0125 per cent was shown to be above. Then at 0.0125 per cent $PbSO_4$ the latter again behaves like $FeSO_4$ in that definite stimulation follows the toxicity of the previous concentration, but while the stimulation of the $FeSO_4$ at a similar concentration is very marked it is only slight in the case of $PbSO_4$. Then, again, at the further concentration of 0.15 per cent $PbSO_4$, the latter acts entirely differently from all the other salts in that it becomes markedly toxic again.

The idiosyncracies of $PbSO_4$ as regards the nitrifying flora of a soil are even more numerous than those of $FeSO_4$. However, it must be borne in mind that $PbSO_4$ is a very insoluble salt and while the other salts are capable of being added in solution to the cultures, $PbSO_4$ has to be mixed with the soil as thoroughly as possible in its dry state. This fact would account for a poor distribution of the salt through the soil mass and thus indicate one possible cause of the extremely erratic behavior of $PbSO_4$ in nitrification cultures. The fact, however, that $FeSO_4$ and $PbSO_4$ behave alike at a concentration of 0.1 per cent would seem to be more than a mere coincidence, and some of the speculations made above with respect to $FeSO_4$ and its behavior may have some cogency here. The most striking fact, however, gleaned from the series of cultures with $PbSO_4$ is that the latter possesses a stimulating power toward the nitrifying flora. Considering the well-known toxic powers of lead with respect to other organisms, this fact assumes considerable importance. In all cases, of course, the stimulating powers of the other salts are much greater than that of $PbSO_4$.

General Discussion

The experimental data above submitted constitute the first series of systematic experiments dealing with the effects of copper, zinc, iron and lead on the ammonifying and nitrifying flora of the same soil. Once more we are confronted by the striking differences between the physiological characteristics of the ammonifying and the nitrifying flora of soils. Other similar

cases, though not so marked, have been commented on elsewhere by one of us (see literature above cited). While the sulfates of all the metals named are toxic to ammonification, and in very low as well as very high concentrations, ranging from 50 parts per million to 2500 parts per million, they manifest a relatively low toxicity for the flora concerned in that phase of nitrogen transformation. On the other hand, all the same metals exercise, in a large variety of concentrations, a marked stimulating effect on the nitrifying flora. That differences of such magnitude should exist between two flora of the soil, one being presumably dependent for its raw material on the other, is, to say the least, amazing and not wholly explicable. Since, however, nitrification in the broader sense of the term represents the algebraic sum of the activities of all forms of nitrogen-transforming bacteria in the soil, it would appear from our results that the net effect of the activity of the metallic salts tested is to insure a larger nitrate supply in the soil. This must be so, since in the most extreme cases above studied ammonification is depressed only to an extent which lowers the total ammonia yields below that of the salt-free soil by about 30 per cent, whereas nitrate production is frequently enhanced in efficiency by the presence of the same metals to an extent which doubles the yield of a normal soil flora.

Our findings would therefore render much easier the explanation of the oft-noted stimulating effect of copper, in particular, and metallic salts in general (in proper concentrations), on the higher plants, regarding which, also, we have accumulated considerable data. If a larger nitrate production in the soil follows the application of a metallic salt as above shown, the nitrogen nutrition of plants must go on with greater facility and adequacy: hence increased growth.

As above intimated, the underlying causes of our very interesting results are not easy to discover. In addition, however, to the speculations on that subject which we make, and which we recognize for more reasons than one as far from satisfying, we have obtained some experimental evidence on the point in question which possesses more cogency. It appears from these that water absorption is hastened by germinating seeds and young

plants in the soil in the presence of copper. Why might not a similar effect be exercised by metals as regards the nitrifying bacteria which, physiologically speaking, in every way resemble the higher plants much more than the other soil flora?

CONCLUSIONS

1. Copper, zinc, iron and lead exercise toxic effects on the ammonifying flora of a sandy soil from Southern California in all concentrations, ranging from 50 to 2500 parts per million of their sulfates.

2. Such toxicity is relatively small, however, and is more marked at concentrations below 0.1 per cent than above it, in most cases.

3. The metals named exercise no stimulating effect on the ammonifying flora at any concentration.

4. Copper, zinc, iron and lead exercise marked stimulating effects on the nitrifying flora of the same soil and frequently more than double the normal nitrate yield.

5. The same metals may in very small concentrations exercise toxic effects on the nitrifying flora or no effect at all, but they are markedly stimulating at much higher concentrations.

6. With the one exception of lead sulfate, the metals named showed very marked stimulation for nitrification even at 0.15 per cent, the highest concentration employed.

7. Comparisons are given of our work with that of one or two other investigators on portions of the same subject. In some cases our results confirm previous work. In other cases they are wholly at variance with it.

8. A brief discussion is given of possible causes for the effects of the metallic salts under consideration on the soil flora.

9. Fuller consideration will be given in another paper to the theoretical phases of the subject treated above.

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